

AN INTERSPECIFIC HYBRID IN ALLIUM<sup>1</sup>S. L. EMSWELLER<sup>2</sup> AND H. A. JONES<sup>3</sup>

## INTRODUCTION

PORTER AND JONES,<sup>(5)</sup> testing several varieties of *Allium cepa* L. and other species of the same genus, found that the Nebuka, a type of *A. fistulosum*, was markedly resistant to pinkroot, caused by *Phoma terrestris* Hansen, and showed no indication of injury when grown continuously for several years in heavily infested soil. Felix<sup>(2)</sup> has also reported that varieties of *A. fistulosum*, namely Winterhecke and White Welsh as well as different strains of Nebuka (Natsu-negi Nebuka, Sinju negi Nebuka, Tokyo-Nebuka, Itwatsuki), are resistant to pinkroot. In addition, he states that the different strains of Nebuka are highly resistant to smut, caused by *Urocystis cepulae* Frost: although under favorable conditions, infection frequently averaged 10 to 20 per cent in the cotyledon stage, it always fell to 0.5 per cent or less in later counts. Anderson<sup>(1)</sup> also reports that the variety Winterhecke is resistant to smut. In a letter dated December 6, 1932, Professor A. G. Newhall of Cornell University states that seedlings from Nebuka seed grown at Davis, California, were practically immune to smut.

In a recent paper, Jones, Bailey, and Emsweller<sup>(4)</sup> have shown that the Nebuka onion is also resistant to thrips, having a smaller number per plant than any other onion variety except White Persian, which it somewhat resembles in foliage characters, especially the circular leaves, spreading habit of growth, and long sheath region.

The various types of *Allium fistulosum* have little commercial importance in America; but their resistance to pinkroot, smut, and thrips would be very valuable characters to incorporate in any of the *A. cepa*

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varieties. With these objectives in view, crosses between the two species were made in the spring of 1931. For the first cross a strain of Yellow Globe Danvers (pedigree, 15-108-1), inbred for two generations, was used as the female parent; and Nebuka (pedigree 37-1) was used as the male parent. Only 7 seeds were secured from several hundred hand pollinations, and these were planted as soon as mature. Although several

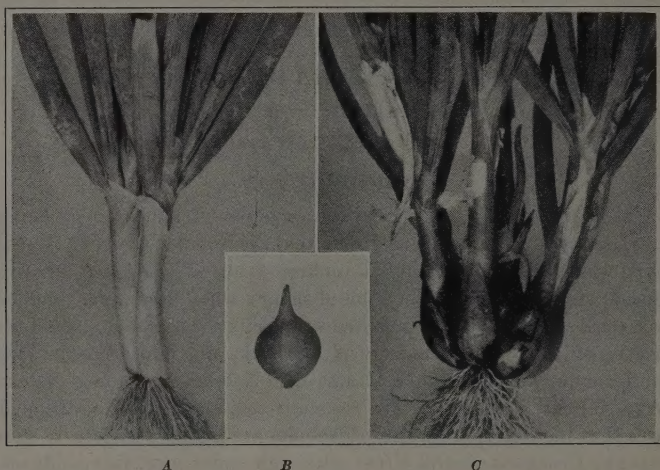


Fig. 1.—A, *Allium fistulosum* (type, Nebuka); B, *Allium cepa*, variety Yellow Globe Danvers, pedigree 15-108-1; C, the  $F_1$  hybrid. Photographed September 9, 1931.

germinated, only 1 seedling survived. This grew very rapidly during the summer of 1931, forming a number of basal subdivisions (fig. 1), which were separated and replanted on September 9.

A second cross was made in 1933 between the variety White Persian (*Allium cepa*) and Nebuka. From the 110 seeds secured, only 25 seedlings survived. The White Persian variety, obtained from Persia by the United States Department of Agriculture Division of Foreign Plant Introduction, has been described elsewhere.<sup>(4)</sup> This hybrid was particularly interesting because of the ease with which a large number of seeds were secured and because of their precocious germination within the unripened ovary. While being removed from the ovary, many of the germinating seeds were killed by injury to the emerged radicle. This phenomenon was probably not caused by environmental conditions, for when other umbels of the same White Persian plant were selfed and open-pollinated, the seed behaved normally. Possibly Nebuka is more



closely related to White Persian than to other commonly cultivated varieties; and, as stated above, the two resemble each other very closely in certain characters.

In the spring of 1934, flies were used to make crosses between Nebuka and Crystal White Wax, Yellow Bermuda, and several other *cepa* varieties. In the Crystal White Wax  $\times$  Nebuka cross, 135 seeds were secured; in the Yellow Bermuda  $\times$  Nebuka cross, 175 seeds. The seed set readily in both crosses. At present a large number of the hybrids are growing vigorously.

The hybrids mentioned above were secured by controlled pollinations, made either by hand or with flies. Hybrids between the two species have also been secured by planting adjacent cultures and collecting open-pollinated seed. In 1932, Nebuka and Australian Brown were planted side by side. In 1933, 32 species hybrids were found among the plants grown from the Nebuka seed, on an area of about  $\frac{1}{16}$  acre. They were recognized in the field by their vigor and semicircular leaves, which were typical of the hybrids produced under controlled conditions. The second year, the floral characters and sterility of these plants fully confirmed their hybrid nature. Ten of these hybrids were divided and planted to be used for further study and for backcrossing to each parent.

In 1933, Nebuka (*Allium fistulosum*), and California Early Red, (*A. cepa*, 21-22-1) were planted close together and open-pollinated seed was collected. In a population of 836 plants grown from seed of the Nebuka parent, 17 hybrids were found, whereas among approximately 5,000 plants grown from seed of the California Early Red parent, no hybrids appeared. Apparently, therefore, these species cross in the open, but more readily in one direction.

### BEHAVIOR OF THE HYBRID

Thus far, hybrids between Nebuka and Yellow Globe Danvers, Australian Brown, White Persian, and California Early Red have been grown through their vegetative cycle, and the first three have also been carried through at least one season of flowering. As regards certain of the following characters, all behaved alike.

*Bulbing*.—The Nebuka types are nonbulbing and, as they grow at Davis, display no swelling at the base of the sheath region. The hybrids of Nebuka with *Allium cepa* are slightly bulbing (fig. 2).

*Life Cycle*.—Nebuka is a perennial type; although seed is produced in abundance, the plants continue to form divisions at the base, and these can be used for propagation. The varieties of *Allium cepa* are usually grown as biennials, a bulb being produced the first year and seed the second. The hybrids between the two are perennials; the tops remain

erect, and the plants continue to grow as long as weather conditions are favorable. The single Yellow Globe Danvers  $\times$  Nebuka hybrid originally secured has been increased by vegetative propagation until at present 208 plants are growing in the field. The other hybrid plants are also being increased by this method.



Fig. 2.—Hybrids of Yellow Globe Danvers (15-108-1)  $\times$  Nebuka showing the extent of bulbing in the F<sub>1</sub>. Photographed August 13, 1934.

*Date of Flowering.*—In the fall of 1931, the Yellow Globe Danvers  $\times$  Nebuka hybrid and both parents were set side by side in the open field so that earliness of flowering and length of flowering period might be compared. The following spring the first Nebuka flowers were open on March 19, those of Yellow Globe Danvers on May 8, and those of the hybrid on April 10. Another comparison was made in 1933, when the first flowers of Nebuka, Yellow Globe Danvers, and the hybrid opened on March 12, May 14, and April 11, respectively. Nebuka flowers are always first to open, and Yellow Globe Danvers last, with the hybrid opening midway in time between the two parents. Nebuka usually begins to bloom early in March and, as a rule, continues until late July or early August; Yellow Globe Danvers has a much shorter flowering period beginning about the first of May and finishing early in July. The hybrid begins to bloom about a month after Nebuka and, as a rule, continues fully as long.





Fig. 3.—Early and late flowering stages: A and B, *Allium cepa*; C and D, *Allium fistulosum*; and E and F, the *cepa* × *fistulosum* hybrid.

The Nebuka  $\times$  Australian Brown and the White Persian  $\times$  Nebuka hybrids bloomed for the first time in the spring of 1934. They also were intermediate in time of flowering and in length of flowering season.

*Type of Inflorescence.*—In Yellow Globe Danvers of *Allium cepa* (fig. 3 A, B) anthesis occurs over the entire inflorescence throughout the



Fig. 4.—Flowers at different stages of development: A, *Allium cepa*; B, *Allium fistulosum*; and C, the hybrid. At 1 and 2, the inner whorl of stamens is elongated; at 3, the inner whorl has completed its growth, and the outer whorl is elongating; 4, the inner whorl has shed its pollen; 5, all pollen has been shed; and 6, stigmas are receptive.

flowering period. In Nebuka, *A. fistulosum* (fig. 3 C, D), the terminal flowers open first, and anthesis proceeds progressively toward the base. At the tip of the umbel, seed may be practically mature, while toward the base buds are just opening. In flowering habit the hybrid (fig. 3 E, F) is intermediate between that of the parents. The terminal flowers are the first to open (fig. 3E) although this process is less clearly defined than in *A. fistulosum*; then they mature progressively toward the base. Thereafter, anthesis occurs over the entire inflorescence (fig. 3F), as is the habit of Yellow Globe Danvers and other varieties of *A. cepa*.

*Flower Habit.*—In *Allium cepa* the flower is fully expanded at anthesis. In *A. fistulosum* the perianth remains erect. In this character the hybrid resembles the *fistulosum* parent very closely (fig. 4).



*Fertility*.—All the hybrids that have bloomed have been practically self-sterile, though several hundred inflorescences have been isolated under paper as well as cloth bags, with flies as pollinators. In the White Persian  $\times$  Nebuka hybrids three  $F_2$  seeds have been secured. Under open-pollinated conditions, however, five seeds were obtained in 1933 from a large group of the Yellow Globe Danvers  $\times$  Nebuka hybrids. These are probably backcrosses to several *Allium cepa* varieties growing nearby. In 1934 they formed bulbs of various sizes, shapes, and colors. When the Yellow Globe Danvers  $\times$  Nebuka hybrid was used as the maternal parent in controlled backcrosses, seed was never secured. This hybrid has, however, functioned as a pollen parent in backcrosses to both *A. cepa* and *A. fistulosum*. Its behavior is rather unusual, for in most instances species hybrids have been more highly backcross fertile when used as the maternal parent. If only a small per cent of the gametes are functional, however, they probably occur more frequently in the sperm cells because of the very large number produced as compared with the egg cells. Since at least some of the eggs and pollen grains are functional, one might reasonably expect that under favorable conditions some  $F_2$  seed should be secured.

*Resistance to Pinkroot and Smut*.—The Yellow Globe Danvers  $\times$  Nebuka and the Nebuka  $\times$  Australian Brown hybrids have been grown in the field on soil heavily infested with the pinkroot organism. Though the roots show some pink, the plants apparently are not checked in their growth. The variety Australian Brown has proved very susceptible to pinkroot; yet the hybrid grew vigorously on sick soil throughout the season. Even though some roots may be killed by the pinkroot organism, the plants are able, apparently, to supply new ones fast enough for good growth. The hybrids have thus far not been tested for smut resistance.

*Leaf Shape*.—In the Nebuka varieties the leaves are circular in cross section, whereas in Yellow Globe Danvers, Australian Brown, and California Early Red the leaf blade is semicircular. In the hybrid involving these varieties, the *Allium cepa* type of leaf is dominant. The White Persian, as noted above, has a leaf blade that is circular in cross section, closely resembling that of *A. fistulosum*. As expected, the leaf blade of the hybrid is similar, giving this cross a distinctive appearance.

*Vigor*.—Under similar conditions, the hybrids grow considerably taller than either of the parents.

*Stability*.—These hybrids, though practically sterile, are very vigorous and multiply rapidly by subdivisions at the base. Under natural conditions, they would probably be able to increase and perpetuate themselves. In this respect they resemble garlic (*Allium sativum*), a fully accepted species, which is completely sterile and reproduces en-

tirely by division of the bulb. The widespread distribution of garlic has probably been accomplished by man because of its economic importance.

*Backcrosses.*—Fortunately the hybrids have proved somewhat fertile in backcrosses to both species, but only when used as the pollen parent. In 1933, backcrosses were obtained to both *Allium cepa* and *A. fistulosum*; and backcross seedlings are now being grown. It is very likely that as backcrossing continues, fertility will increase, and it will be possible to secure a sufficiently large population to make selections for resistance to pinkroot and smut.

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MEIOSIS IN ALLIUM FISTULOSUM, ALLIUM  
CEPA, AND THEIR HYBRID

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# MEIOSIS IN ALLIUM FISTULOSUM, ALLIUM CEPA, AND THEIR HYBRID<sup>1</sup>

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A PRECEDING PAPER<sup>(4)</sup> discussed the general morphology of several varieties of *Allium cepa* L., *Allium fistulosum* L., and their hybrids.

Because of the high degree of self-sterility in the hybrid, cytological studies were begun in 1932 in the hope that they might explain the cause of sterility and possibly indicate whether a stable derivative combining the desirable characters of the two species might be secured. These investigations have now been completed on the Yellow Globe Danvers variety of *Allium cepa* (pedigree 15-108-1), on a strain of *fistulosum* (37-1), and on their hybrid.

## METHODS

To secure material containing pollen mother cells at desirable stages, an anther was first crushed in aceto carmine and examined under low power. If suitable division figures were found, the remaining five anthers of a flower were removed and fixed, for at least 24 hours, in a solution of 25 per cent acetic acid and 75 per cent absolute alcohol. They were then transferred to 80 per cent alcohol, where they may be kept indefinitely. Material stored for four months and then stained has given better differentiation between chromatin and cytoplasm than material used a few days after fixing.

When preparations were made from fixed material, an anther was placed on a slide and covered with a small drop of aceto carmine. The aceto carmine had to contain a considerable amount of iron; otherwise the chromosomes were stained very weakly, and differentiation was not sharp. A curved, blunt needle was used to press out the pollen mother cells, and the anther wall and all other débris were carefully removed before the cover slip was added. All excess aceto carmine was wiped off, the slide examined under low power, and its preparation completed under observation. Usually the cells were found in masses scattered over the slide. If the cover slip was tapped lightly with a bent needle, the pollen mother cells were separated from one another; and further care-

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ful tapping burst the cell wall, allowing the cytoplasm and chromatin to float free. When the slide was heated several times before being tapped, the cell walls were more easily ruptured, the cytoplasm became clearer and was flattened out, and the chromosomes stained more deeply. The edges of the cover slip were then sealed with a gum mastic and paraffin mixture to prevent leakage and preserve the slide until it could be examined. The chromatin in the pollen mother cells treated in this way was rarely injured. So many excellent normal figures were available that any questionable ones could be discarded. Unusually fine preparations of early prophase chromosomes were secured, and especially desirable slides were converted into permanents after the method suggested by McClintock.<sup>(9)</sup> Smears of pollen grains were prepared in the same way.

Camera lucida drawings of the chromosomes were made by using a Zeiss microscope with binocular attachment, 15 $\times$  compensating oculars, and a 90 $\times$ -1.4 apochromatic objective. The photomicrographs were taken with a Zeiss photomicrographic apparatus, several different sets of oculars and objectives being used. In each instance, the magnification is recorded with the photograph.

### CHROMOSOME MORPHOLOGY

The chromosome number of both *Allium cepa* and *A. fistulosum*, as reported by several investigators, is 16. Though the chromosomes of the former have long been a favorite subject of research, opinions as to their morphology have differed considerably. Schaffner<sup>(13)</sup> shows in his drawings some chromosomes with median attachments; Neme<sup>(11)</sup> indicates by his figures that they are median, terminal, and subterminal; Miyake<sup>(10)</sup> shows median attachments, apparently for all; Lündegårdh<sup>(8)</sup> in one figure illustrates both median and submedian; and Taylor<sup>(15)</sup> reports a subterminal pair of chromosomes with a satellite attached to the short arm. The chromosomes of *fistulosum* have not received such widespread attention. Ishikawa<sup>(6)</sup> described practically all as having median constrictions, while Strasburger<sup>(14)</sup> gives figures showing both median and submedian. Such wide diversity of observation is probably caused by the entanglement of the large chromosomes, which in somatic plates usually makes it very difficult to observe them free from one another in the cell.

In the present study the morphology of the chromosomes was determined in the pollen grain by observing plates of the first division of the microspore. As shown by Levan,<sup>(7)</sup> the chromosomes of *Allium* are usually shorter, plumper, and better spaced at this time than during mitosis of somatic cells. In addition, their morphology can be studied much more easily, since only one chromosome of each pair is present.



In figure 1, the chromosomes of the two species are so arranged that those morphologically similar are opposite each other. For convenience in discussion, each is given a number, prefixed with the first letter of the name of the species to which it belongs. This arrangement does not mean that those placed opposite one another are genetically homologous.

The length of any individual chromosome in the species when measured in different cells is not constant. This was also found by Levan<sup>(7)</sup>

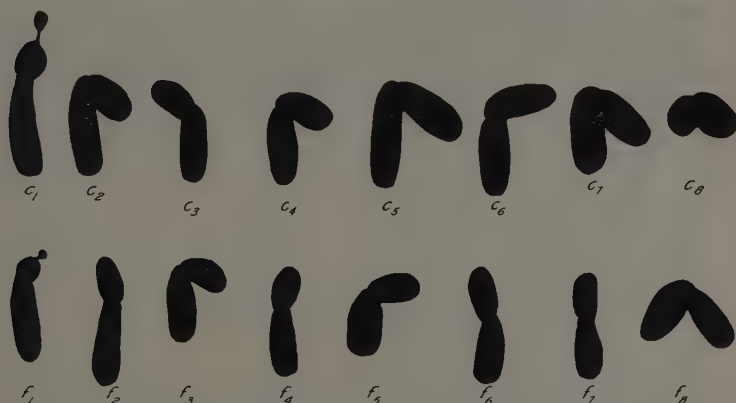


Fig. 1.—Chromosomes of *Allium cepa* (upper) and *Allium fistulosum* (lower) (approx.  $\times 2500$ ).

in *Allium*, by Darlington<sup>(3)</sup> in *Tradescantia*, and by Hollingshead<sup>(5)</sup> in *Crepis*. The variation in total length of the same chromosome might be caused by improper fixation and different stages of contraction. Besides, chromosome size may vary in cells of different tissues, as shown by Hollingshead<sup>(5)</sup> in *Crepis*.

With these difficulties in mind, a method was developed that entirely avoided total chromosome length. Each chromosome was given an index number secured by dividing the length of the short arm by that of the long arm. The measurements were secured from drawings made by projecting metaphase plates of the first microspore division upon a sheet of paper with a Zeiss microprojector. The unit of measure employed was a millimeter. Only those plates were used in which all the chromosomes were flattened out. The constriction region is assumed to be located at a definite point on the chromosome. It should, then, at least at late metaphase, divide the chromosome into two arms, from which a constant index figure can be calculated, as described above. A chromosome with an exactly median constriction would have arms equal in length and an index of 1.0. As the constriction region became more and more terminal,

the index would approach 0. A total of 88 chromosomes in 11 pollen grains of *Allium cepa* and 72 chromosomes in 9 pollen grains of *A. fistulosum* were measured in this manner. The index figure for each of the 8 chromosomes in a pollen grain was computed and arranged in order from most nearly terminal to median. In this way the chromosomes of each pollen grain were placed into one of 8 types (the haploid number of each species). When all the chromosomes were so arranged, in no instance was a deviation of more than 0.08 found within any lot of the same type of chromosome. Occasionally an individual chromosome of one type (table 1) showed an index figure slightly larger than some in the type above or slightly smaller than some in the group below.

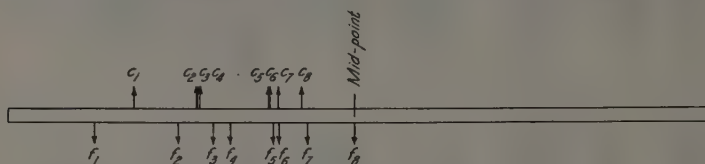


Fig. 2.—Showing relative positions of insertion regions for *Allium cepa* and *A. fistulosum* chromosomes.

Morphological differences between the chromosomes of the two species are very slight, with the exception of chromosome  $c_8$  and  $f_8$  (fig. 1). That slight differences probably do exist, however, is brought out by the index-number method just described, and is shown graphically in figure 2. The constriction regions for each *cepa* chromosome are shown above and those for *fistulosum* below the line. Since total length is ignored, all sixteen constriction regions can be depicted on one line. The average lengths of the chromosomes and their index numbers are given in table 1. When studied in conjunction with the diagram, this table indicates that differences between some members of the *cepa* genom are very small. Because, for example,  $c_2$ ,  $c_3$ , and  $c_4$  are so very similar, it is practically impossible to be sure that actual differences in length of chromosome and position of insertion region exist. In  $c_5$  and  $c_6$  there is also a very great similarity; but  $c_7$ , which has an index number very close to them, is considerably shorter and is easily recognized. The smallest chromosome,  $c_8$ , with the most nearly median constriction, can be readily distinguished from the others, as can  $c_1$  because of its conspicuous satellite.

The differences between members of the genom in *fistulosum* are more pronounced than in *cepa*. The satellited chromosome  $f_1$  is of course easily recognized. The next most terminally constricted chromosome,  $f_2$ , is considerably longer than  $f_3$  and  $f_4$ , which are so similar in length and index number as to be virtually indistinguishable. The chromosomes  $f_5$  and  $f_6$ ,

though very close in index number, differ from each other considerably in length and are less medianly constricted than  $f_7$ . The last member of the set,  $f_8$ , has equal arms because of its fully median constriction.

A comparison of the chromosomal morphology of the two species indicates that they probably had a common ancestor and now differ from one another as a result of internal chromosomal changes involving gene rearrangement and mutations.

TABLE 1  
AVERAGE TOTAL LENGTHS AND INDEX NUMBERS OF CHROMOSOMES  
OF *CEPA* AND *FISTULOSUM*

Chromosome No.	<i>Allium cepa</i>		<i>Allium fistulosum</i>	
	Length, mm	Index No.	Length, mm	Index No.
1	38.8	0.3661	30.2	0.2479
2	29.5	0.5463	39.5	0.4905
3	31.7	0.5469	29.4	0.5978
4	31.5	0.5594	31.7	0.6424
5	42.4	0.7520	36.8	0.7692
6	38.6	0.7545	29.5	0.7878
7	28.5	0.7864	32.5	0.8678
8	18.4	0.8508	31.6	1.0000
Total	261.2		259.4	

### MEIOSIS IN *ALLIUM FISTULOSUM*

Levan<sup>(7)</sup> has presented a detailed account of meiosis in *Allium fistulosum*. The observations reported in the present paper completely agree with his. The strain of *fistulosum* used in this hybrid is probably very similar to his types 1, 2, or 4.

In very early prophase, such as *A*, plate 1, the chromosomes appear to be synapsing as single threads. The cell represented shows this condition at several points, but especially at *a*, where a half twist is very clear. At this stage it is impossible to follow accurately a single pair of chromosomes, or to count the total number of such twists. The observations in different cells at comparable stages have all shown a similar condition, as does Levan's figure 6. Unquestionably, the threads are paired, since counts have shown from 12 to 14 free ends. In no cell observed could all 16 ends be distinguished, because some chromosome clumping usually occurs at this time.

A later stage (diplotene) is shown in *B* of the same plate. Each chromosome now appears to be composed of two chromatids. This is particularly clear at *a* and *b*, where there is an exchange of partners. The arrows converging at *c* are pointing from what appear to be overlaps, not



chiasmata. As noted by Belling,<sup>(2)</sup> counts of chiasmata frequency very likely include many overlaps of this type. Open loops are already present, and most of the bivalents can be followed from end to end. Careful counts of the number of strands visible in some of the chromosomes, made at similar and slightly earlier stages, are recorded in table 2. In

TABLE 2

NUMBER OF CHROMOSOMES OBSERVED AS SINGLE AND AS DOUBLE STRANDS  
AT ZYGOTENE AND DIPLTENE IN *ALLIUM FISTULOSUM*

Zygotene			Late pachytene to early diplotene		
Cell No.	Single strands	Double strands	Cell No.	Single strands	Double strands
1	5	0	1	1	2
2	11	0	2	0	6
3	2	0	3	0	2
4	2	0	4	0	2
5	4	0	5	0	3
6	1	0	6	0	2
7	3	0	7	1	2
8	2	0	8	0	2
9	3	0	9	0	1
10	3	0	10	0	1
11	7	0	11	0	2
12	8	0	12	0	2
Total	53	0	Total	2	27

two cells at diplotene, both split and unsplit chromosomes were observed. Probably, therefore, the splitting of the different chromosomes does not occur simultaneously or at the same rate. This situation is also to be inferred from figures 7, 8, 9, 10, 11, and 12 of Levan. Succeeding figures by the same author clearly show four chromatids.

A group of diplotene bivalents is shown in plate 2A. The two chromatids of each chromosome are visible at certain points, and the number of chiasmata per bivalent is greatly reduced from that of plate 1B. Terminal chiasmata can be seen at four of the sixteen ends.

At diakinesis, plate 2B, chiasma frequency is still further reduced; and here, too, in many places, each chromosome is seen to be composed of two chromatids. No terminal chiasmata are present.

A polar and a sideview of IM are shown in plate 2C and plate 3A. These are practically identical with metaphase figures of Levan and show the localization of chiasmata at the constriction region. Although there appears to be a single chiasma in each bivalent, there are really at least two—one on each side of the constriction, as is readily observed when the paired chromosomes begin to separate. Other instances of localization of chiasmata are thoroughly discussed by Levan.<sup>(7)</sup>

In both *cepa* and *fistulosum* the separation of paired chromosomes appears to result from an external force rather than a repulsion between the chromosomes (plate 3, *B* and *C*). At the constriction region of several of the bivalents just beginning to separate, a strong pull is apparently being exerted from outside the chromosome, resulting in the drawing out of the matrix to a sharp point. In the anaphase, the configurations of the chromosomes also support this hypothesis. The pulling out of the chromosome matrix can be seen here also (plate 3*B*). At this time it does not seem likely that any repulsion between chromosomes could account for this situation. Most of the bivalents of plate 2*C* seem to have homologous arms well spread apart, but at least three do not show this condition. The spreading of the arms might be interpreted as caused by repulsion; but the writers, after viewing many metaphase plates, are inclined to believe that it is probably fortuitous. The chromosomes are held together only at the insertion region, and the arms float free in the cytoplasm. They may be separated or may lie side by side. Judging, also, from plate 5*B*, the free arms that are separated are not repelling one another, since the end of each is bent back toward the other. It is not to be expected that the free ends of chromosomes which are held together at some interstitial region will either be spread fully apart or remain close together. There is evidently a range from lying close to one another to being fully spread—a range fairly well represented in the bivalents of plate 2*C*.

In the anaphase, the split that occurred at pachytene is completed; and the two chromatids of each chromosome are held together only at the constriction region (plate 3*B*). The split that will be completed in the first division of the pollen grain is not yet visible. Only the satellited chromosome assumes a J-shape; all others, because of their more or less median constrictions, are V-shaped.

The formation of dyads and tetrads is entirely normal. In no instance were micronuclei observed. The pollen grains appeared normal; and eight chromosomes were found in all grains in which metaphase figures were seen.

#### THE RELATION OF THE NUCLEOLI TO THE SATELLITED CHROMOSOMES IN *ALLIUM FISTULOSUM*

The question of the function of the nucleolus is entirely beyond the scope of this paper. During our observations, however, there appeared to be a constant and striking association between the nucleolus and the satellited chromosomes. Since there has been some doubt as to the existence of such an association, our observations on this phenomenon in *Allium fistulosum* are reported. In plates 4 and 5, *A* and *B*, this associa-

tion is clearly shown. In plate 5A the two satellites are lying on the nucleolus; and in plate 5B this attachment is particularly clear. Though the satellited chromosomes cannot be recognized in very early stages, this difficulty is not encountered later, when these chromosomes are easily identified because of their subterminal constriction, at which the chiasmata are localized. In this study we have never seen a single instance at diplotene or diakinesis in which the nucleolus or nucleoli were not associated with the satellited chromosomes. Very probably, this association has existed from the time the nucleolus was reconstituted from the preceding interphase.

### MEIOSIS IN *ALLIUM CEPA*

The early stages of meiosis in the strain of *Allium cepa* used in this study resemble those of *A. fistulosum*. The early prophase chromosomes appear to be single threads, and not until diakinesis does any difference between the two species become apparent. At this stage in *A. fistulosum* (plate 2B) some localization of chiasmata already appears—a condition not found in *A. cepa*. The metaphase bivalents of the two species differ considerably (plates 2C and 3C). In *A. cepa* the chiasmata are all terminal, resulting in two general types of bivalent configurations—rings and rods. In some cells all eight show terminal attachment of both ends; and the separation of both ends is not simultaneous (plate 3C). This situation is also a condition of localization, differing from *A. fistulosum* in being terminal rather than interstitial. Meiosis in *A. cepa* is not completely described here, since it parallels *A. fistulosum* in all stages, except that at metaphase the chiasmata are not localized at the constriction region.

### MEIOSIS IN THE HYBRID

In the early meiotic prophase of the hybrid, the chromosomes appear to be single threads as in the two parents. In these stages it has not been possible to distinguish any irregularities. At late pachytene and diplotene, however, abnormalities are readily found. Chromosomes of unequal lengths are paired; and sometimes those approximately equal conjugate so that each has a long unpaired arm extending well beyond the end of the other. At diakinesis the bivalents are usually well spaced, and abnormalities are easily distinguished.

At IM the bivalents resemble those in *cepa* (plate 6A). None with chiasmata localized at the constriction region were observed, the general configuration being rods and rings, even though some of the bivalents are formed by heteromorphic chromosomes.

The frequency of the bivalent formation at IM in the parent species and hybrid is shown in table 3. Univalents are occasionally found in both



*cepa* and *fistulosum*. In the hybrid, unpaired chromosomes are considerably more frequent, the number of bivalents in adjacent cells being variable. In this respect, it resembles hybrids involving species of other genera, a summary of which is given by Avery.<sup>(1)</sup>

At IA separation of paired chromosomes occasionally shows so-called bridges and fragments. A discussion of these phenomena is reserved for a later paper, only clean separations being considered at present. In all the *Alliums* so far examined, the late metaphase and early anaphase bivalents are excellent for studying chromatid relations.

TABLE 3  
CHROMOSOME PAIRING IN THE HYBRID AND PARENT SPECIES

Type of pairing	Frequency		
	F <sub>1</sub>	<i>Allium cepa</i>	<i>Allium fistulosum</i>
8II+0I.....	94	108	107
7II+2I.....	24	3	2
6II+4I.....	9	1	0
5II+6I.....	1	0	0
4II+8I.....	2	0	0
Total.....	130	112	109

Plate 6B shows eight bivalents at late IM in a pollen mother cell of the hybrid. Beginning at the left, the first and sixth show separations in which long and short chromatids are associated. This same situation can also be seen in bivalent 2 of plate 6C. Similar figures have been observed rather frequently. In order to observe details in such bivalents it was necessary to have them well flattened on the slide. The first in plate 6B shows a type of association that appears peculiar until analyzed. The chromatids going to each pole are one short plus one long—a fact that can be determined because the ends of the four chromatids have been spread apart when flattened under the cover slip, so that two were forced to the right and two to the left. In bivalent 8 at the extreme right, the details of chromatid association are not visible, because, when flattened out, the ends of the four chromatids were not separated. Bivalent 1 of plate 6C will also result in a separation of long plus short chromatids. The exchange of partners is especially clear at *a*. In bivalent 2 the association of long plus short is also very clear. This situation is not infrequent in this hybrid. Since chromosomes of unequal lengths are observed to pair, this type of association would be expected to occur at random, according to the arrangement of chiasmata between chromosomes of unequal length, as illustrated in figure 3. Though Belling's<sup>(2)</sup> hypothesis of crossing over is utilized, the same end products would

occur if Sax's<sup>(12)</sup> explanation were used. In *A* (fig. 3), the chiasma nearest each end of the bivalent, formed by the conjugation of (a-a) and (b-b), causes the first division to be reductional at the proximal (left) and equational at the distal (right) end. Then, as a result, the crossover

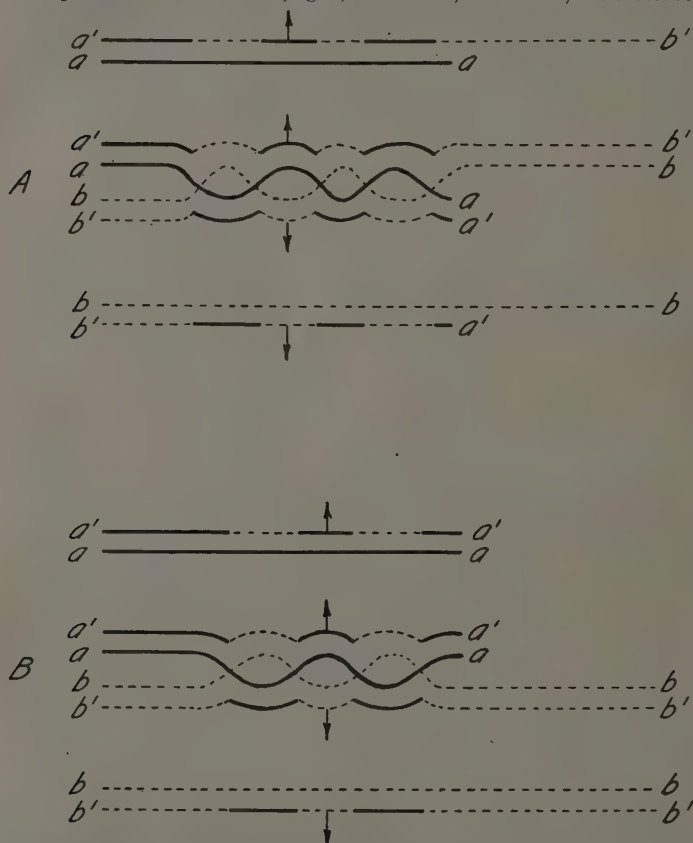


Fig. 3.—Illustrating chiasmata arrangement giving rise to (A) separation of a long plus a short chromatid; and (B) separation of a long plus a long and a short plus a short chromatid.

chromatid (a'-b') goes to the same pole as the shorter noncrossover chromatid (a-a). To the other pole will be segregated the noncrossover (b-b) and the crossover (b'-a'). If, however, the chiasma nearest each end of the pair is as in *B*, the division at both ends will be reductional; and, in consequence, the two short chromatids will be segregated to one

pole, and the two long ones to the other pole. The first division is probably always reductional at the constriction region and both equational and reductional in the two arms, depending on the number of crossovers occurring. Weinrich<sup>(16)</sup> has shown a similar situation in *Phrynotettix*, where bivalents with unequal homologues are seen to separate sometimes with the equal chromatids associated and sometimes with the unequal.

### SUMMARY

The morphology of the chromosomes of *Allium cepa* and *Allium fistulosum* is found to be very similar. A method for securing chromosome index number by determining the ratio of the short to the long arm is presented and affords evidence that the location of the insertion region is constant.

In the very early prophase of each species and their hybrid, the chromosomes appear to be single threads. In pachytene, these threads are seen to be double, and some of the nodes afford clear evidence of exchange of partners (chiasmata), whereas others are very probably overlaps of entire chromosomes.

The separation of paired chromosomes at anaphase appears to depend in part on the operation of some external force that pulls out the chromosome matrix at the insertion region.

At IM in *Allium fistulosum* the chiasmata are interstitial and are localized at the constriction region. In *cepa* they are all terminal. The hybrid has chiasmata very similar to those of *cepa*.

Bivalents formed by chromosomes of different lengths are occasionally found in the hybrid. At IA they occasionally separate with a long plus short chromatid associated. This condition depends upon the arrangement of the chiasmata at the distal and proximal ends of a bivalent.

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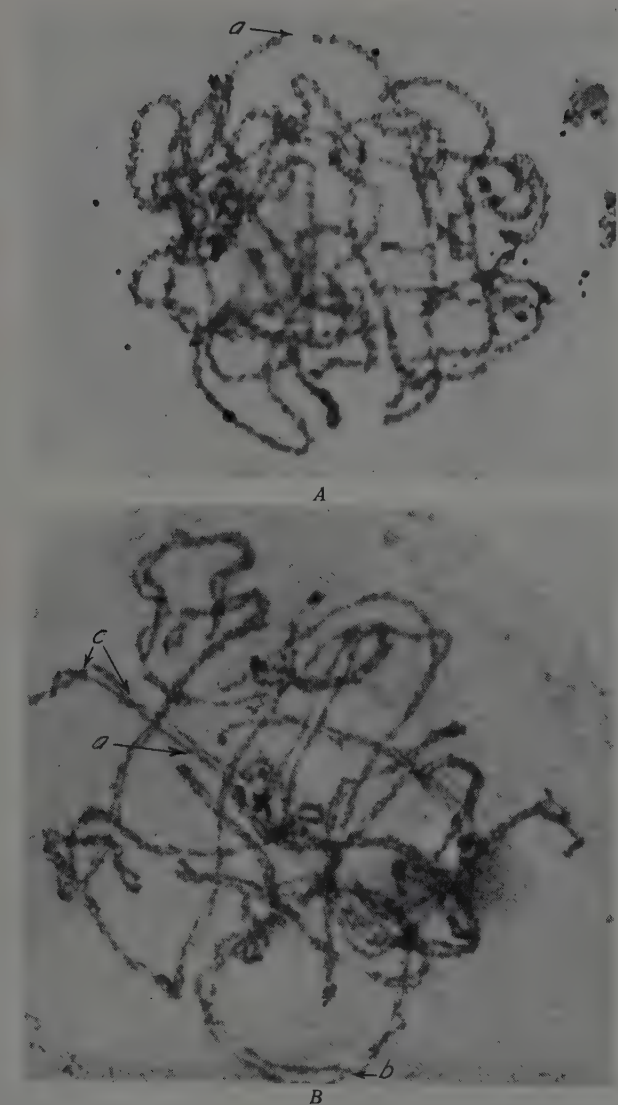


Plate 1.—*Allium fistulosum*: A, zygotene showing unsplit paired chromosomes; B, late pachytene showing split chromosomes and chiasmata. (Approx.  $\times 950$ .)

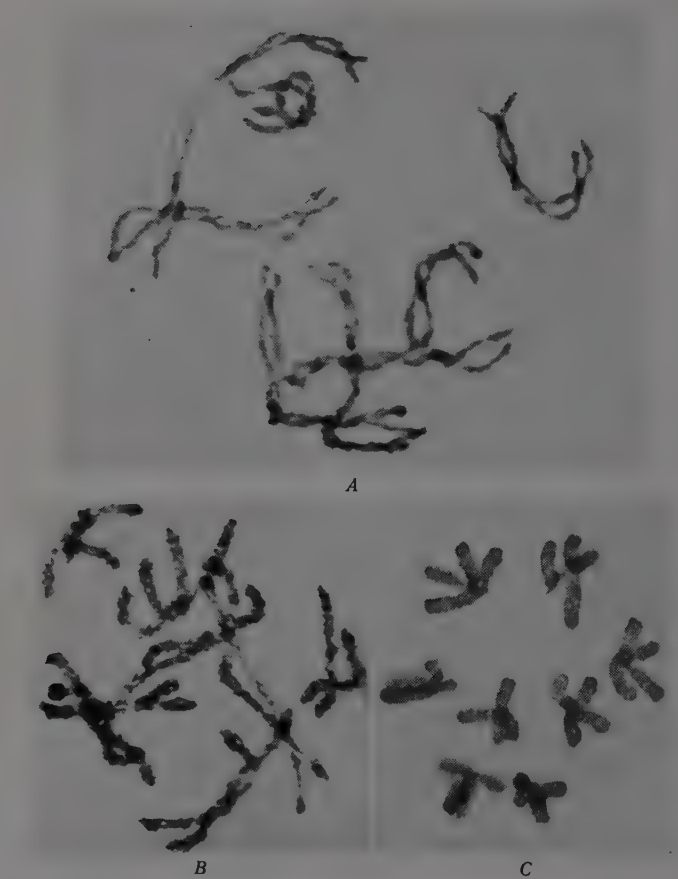


Plate 2.—*Allium fistulosum*: A, late diplotene; B, diakinesis; C, polar view of metaphase showing localization of chiasmata at constriction regions. (Approx.  $\times 950$ .)

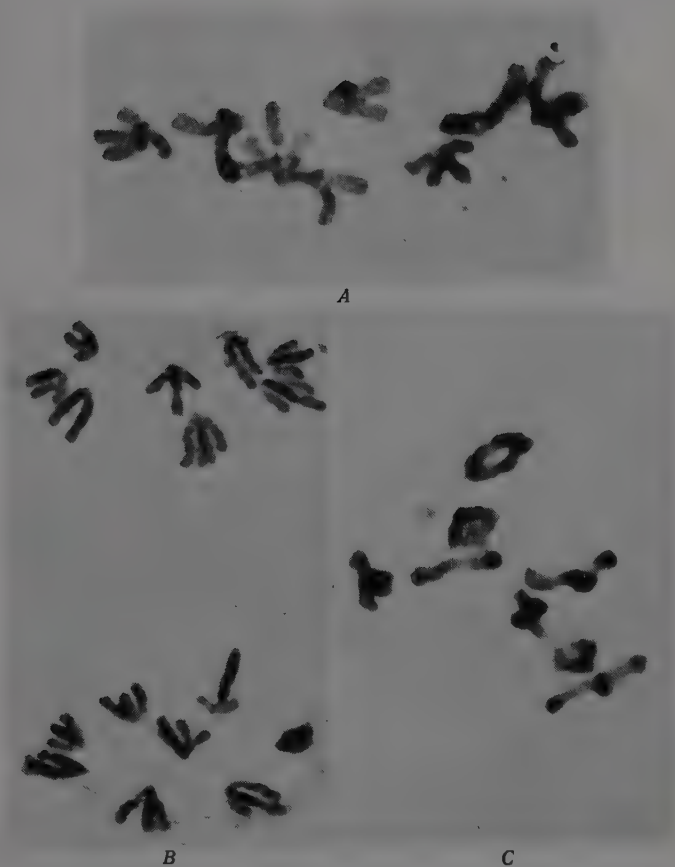
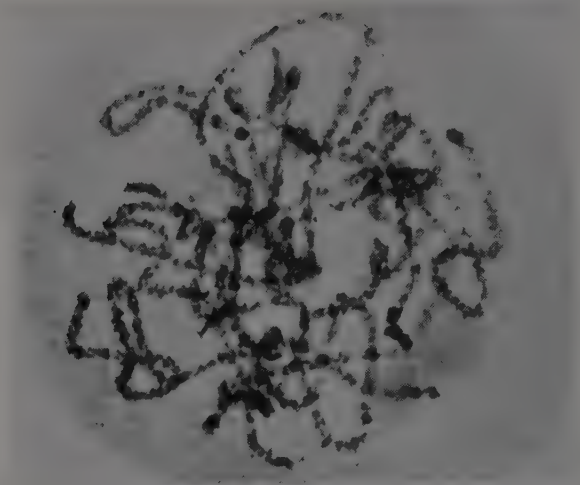
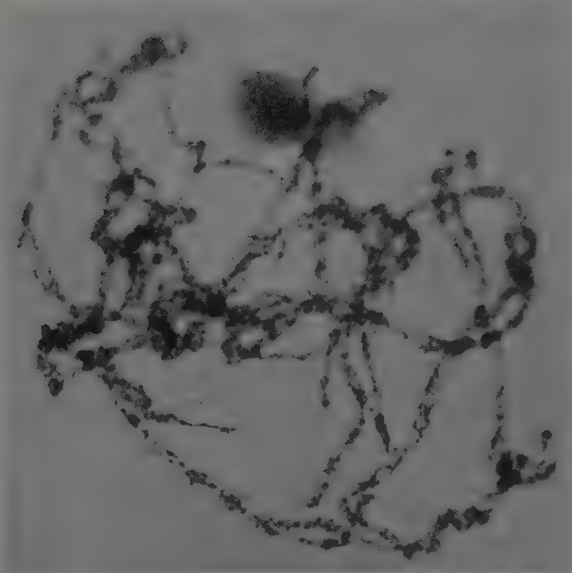


Plate 3.—*Allium fistulosum*: A, side view of first metaphase; B, *Allium fistulosum*, first anaphase; C, *Allium cepa*, first metaphase showing terminal chiasmata. (Approx.  $\times 950$ .)



A



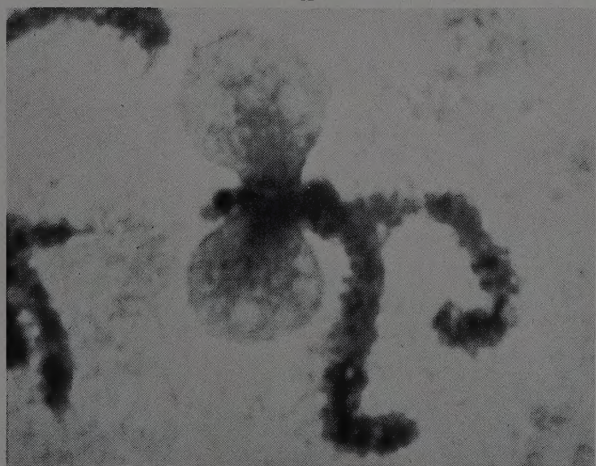
B

Plate 4.—*Allium fistulosum*: A, zygote with nucleolus attached to chromosome; B, early diplotene. (Approx. 950.)



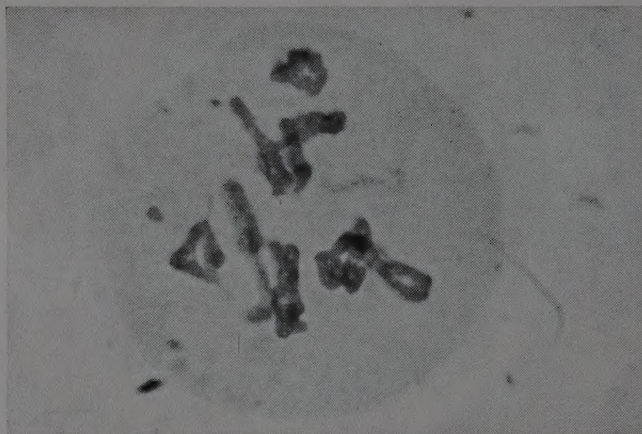


A

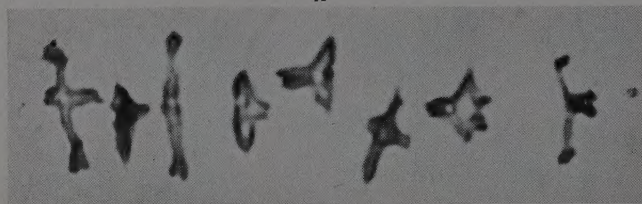


B

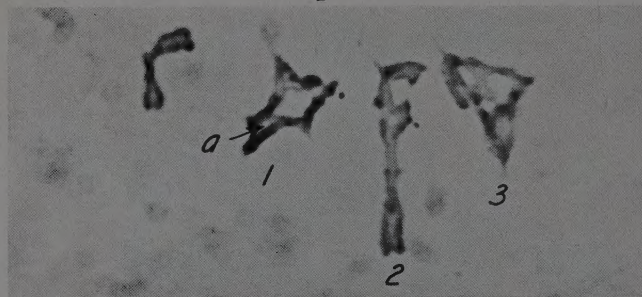
Plate 5.—*Allium fistulosum*: A, late diplotene (approx.  $\times 950$ );  
B, diakinesis (approx.  $\times 1875$ ).



A



B



C

Plate 6.—*Allium cepa*  $\times$  *Allium fistulosum*: A, first metaphase showing terminal chiasmata (approx.  $\times$  950); B, first metaphase with two bivalents showing separation that will result in a long plus a short chromatid to each pole (approx.  $\times$  950); C, three bivalents showing unequal chromosomes separating in 1 and 2 (approx.  $\times$  1250).



